COLD WEATHER PROTECTION FOR SEED

U.S. DEPARTMENT OF AGRICULTURE Science Study Aid No. 6

TEACHER'S GUIDE

This Science Study Aid deals with the problem of cold weather injury to seed. The experiments are designed to show the student how sudden drops in temperature may damage planted seed and affect plant development and how the injury may be prevented.

You can use this Science Study Aid to supplement studies on the germination of seeds, plant injury and diseases, and the affect of environment on plant development.

To carry out the experiments, the student should have a basic understanding of seed and plant structure and be capable of making metric measurements.

The student may go beyond the specific experiments suggested. For example, he could analyze the fat content of a seed by extracting fat with a 2-to-1 chloroform-methanol solution. Or, he could study the development of cotton plants through the bloom and boll stages.

Cold Weather Protection for Seeds was developed by Joan A. Valieant, a junior high school science teacher in the Montgomery County, Md., school system, working with the research staff at the U.S. Agricultural Research Center, Beltsville, Md.

The experiments in this Science Study Aid are based on research done by Dr. Meryl N. Christiansen of the Plant Science Research Division, Agricultural Research Service. They have been tested in the laboratory and in the classrooms of cooperating teachers throughout the country.

BACKGROUND INFORMATION

All plants are more or less sensitive to cold weather. Temperatures near freezing often harm young plants, prevent seeds from germinating, or cause plants to develop abnormally.

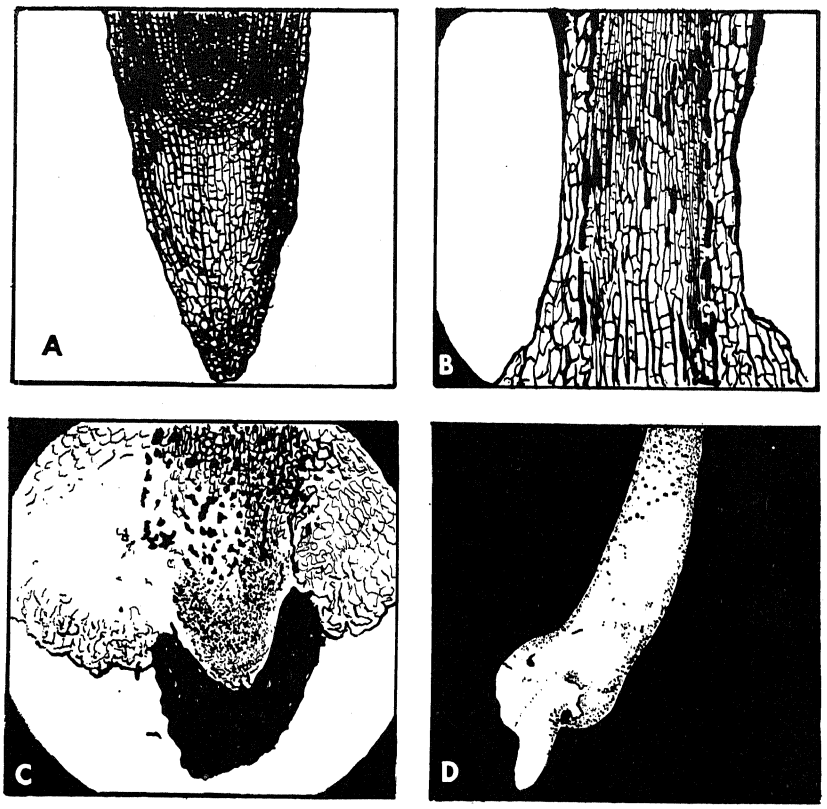
This Science Study Aid deals with a particular kind of cold injury common to cotton and soybeans, two of our most important crops. The injury is called "nub root." It causes the ends of the roots of the affected plants to become swollen and deformed. The roots cannot do as good a job as normal ones in transmitting nutrients from the soil to the plant. Injured plants are generally weaker and less productive than uninjured ones.

Farmers lose millions of dollars every year because of nub root injury to cotton seed. Attempting to avoid this loss, they try to reseed areas where plants suffered damage. Thus, time and labor, as well as money, are lost.

Nub root injury occurs when seeds absorb water at low temperatures—5° to 10° C. (41° to 50° F.). The most obvious damage occurs to the root tip. There is no direct physical damage such as rupture of the cell membrane, but rather there is increasing damage the longer the seed is exposed to low temperature. Apparently, the apical meristem (see figure 1a) is killed or the cell enlargement process is stopped. Factors controlling cell enlargement are probably the source of the problem.

Scientists have found a simple way to prevent nub root injury in cotton seed. Soaking the seeds in water at a temperature of 31° C. (88° F.) before planting will protect them from injury by cold (but not freezing) weather.

The scientists are not yet sure how soaking



ormal and damaged root tips of cotton seedlings. *A*, Longitudinal section of normal seedling root tip; *B*, Section of ıg root damaged by chilling. Note cortical cell disintegration; *C*, Longitudinal section of root cap and meristem of nub root l seedling; and *D*, Intact view of seedling root shown in C.

the seeds prevents injury. The answer may be as explained in the next two paragraphs:

In germinating cotton seed, RNA synthesis, ribosome formation, and development of a protein synthesis system occur during the first 16 hours of water uptake. These processes can be stopped by exposing the seeds to low temperature for 15 minutes. The processes are not permanently stopped, however. Tests have shown that RNA synthesis will begin again after a warm period of 15 minutes following the 15 minutes exposure to cold.

Tests have also shown that metabolic activities increase greatly within a short time after soaking of cotton seeds. This suggests that perhaps pre-soaking the seeds initiates sufficient RNA and protein synthesis to enable the seed to withstand a chilling period and continue cell enlargement.

Figures 2, 3, and 4 show some results of the scientists' investigations.

In figure 2, test seeds were chilled at 5° C. for different lengths of time (horizontal axis of the graph). Then they were germinated at 31° C. for 3 days. The amount of growth is shown on the vertical axis of the graph as a percentage of the control plants' growth. This percentage was calculated from the dry weight ratio of hypocotyl and radicle to the dry weight of the whole plant.

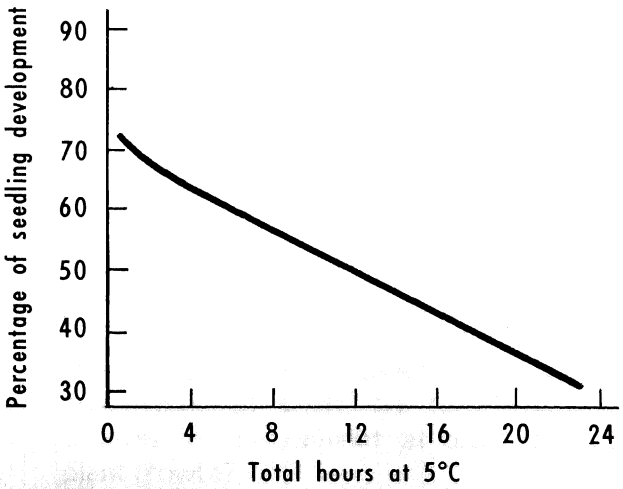


Figure 2.—Seedling development related to exposure to 5° C. temperature during initial period of water uptake.

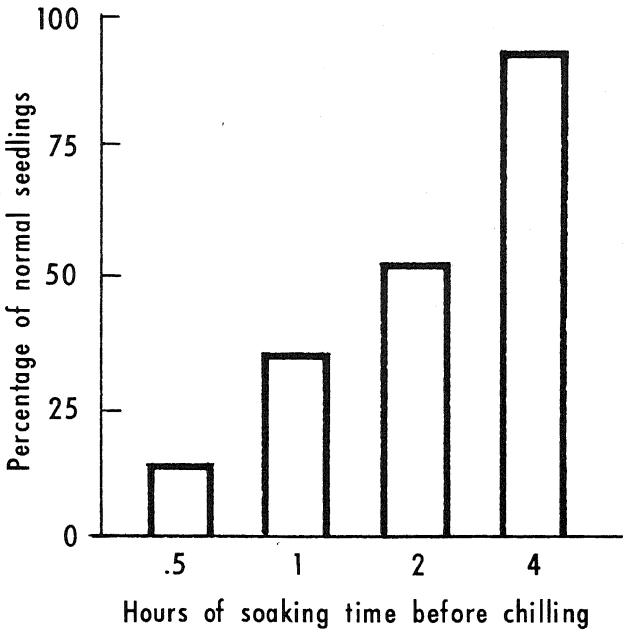


Figure 3.—Seedling development related to time of soaking prior to chilling at 5° C. for 4 hours.

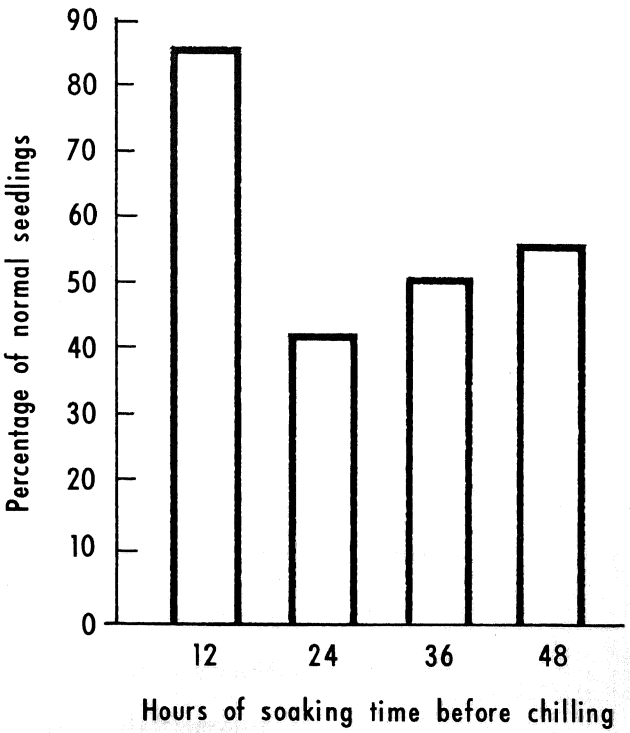


Figure 4.—Seedling development related to time of soaking prior to chilling at 5° C. for 48 hours.

In figure 3, test seeds were soaked in water at 31° C. for different lengths of time. Then they were chilled at 5° C. for 48 hours, and then germinated at 31° C. for 3 days. Note that those soaked for 4 hours had the highest percentage of normal seedlings. Those soaked for shorter periods had more injury to the roots. Normal seedlings were determined by comparing their general appearance with the control plants.

Figure 4 shows the results of seed development after 30 days' growth following 12, 24, 36, and 48 hours of soaking and 48 hours of chilling. Note the results of soaking for increasing lengths of time.

ACTIVITIES

From the experiments in this section, you can learn how sudden drops in the temperature may damage planted seed. Chilling injury may occur when the seeds absorb water at low temperature.

Problem: How can seeds be protected from chilling injury during water uptake at low temperatures?

Hypothesis: Hydration (soaking, of seeds) or germination of seeds, before chilling will prevent injury.

Objective: To determine the effectiveness of a pre-soaking period in preventing chilling injury to seed by exposing cotton seeds to various temperatures after soaking for varying lengths of time.

In doing these experiments, you will need to evaluate seedling development. Discuss the techniques with your teacher.

Try to answer these questions as you do the experiments:

1. At what stage of germination or growth are the plants most susceptible to chilling damage?

2. What is the temperature at which the seed or seedling is most susceptible to chilling damage?

3. What is the nature of damage to the plants?

4. What remedies can be offered to override or prevent the effect of chilling?

5. If chilling has no apparent affect on the germination of seeds, are there significant changes in development at later stages of plant growth?

6. What is the relationship between the amount of moisture absorbed during early stages of germination and subsequent development of the plant?

7. How does the length of time that seeds are chilled affect their growth? For example, after 12, 24, 36, and 48 hours of growth what percentage of seeds are developing at a normal rate in comparison to the control plants?

Note: After observing the results with cotton seeds, you may devise your own experiments with other kinds of seeds, using various temperature ranges, exposure periods, etc.

Materials:

Balance, or scale, accurate in milligrams (mg.)

Beakers, 100 milliliter (ml.) and 600 m.

Cotton seeds. About 500 seeds will be needed for all four experiments. (See box for possible sources of seeds.)

Dissecting needle

Distilled water

Graduated cylinder, 50 or 100 ml.

Incubator

Nutrient soil (3:1:1 compost, fine sand, peat moss. Mixture should be sterilized 6 hours.)

Pots and soil

Rubberbands

Refrigerator

Thermometers

White paper toweling or

Wax paper (household type acceptable)

germination paper. (See box for possible source of germination paper.)

Sources of Materials Needed in the Experiments

1. Germination paper: 12 by 18 inches; $9.75 for 1,000 pieces

   Wax paper: 12 by 18 inches; $10.50 for 1,000 pieces

   Anchor Paper Company
   480 Broadway Street
   St. Paul, Minn. 55101

2. Cotton seeds are available from many sources; the following is a partial list. Seeds may be either treated or untreated, delinted or nondelinted. However, some seed treatment materials are poisonous and treated seed should be handled with care.

   Bridge Enterprises
   Box 113
   Watertown, Mass. 02172

   H. G. Hastings Seed Company
   P. O. Box 4088
   Atlanta, Ga. 30302

   John D. Rogers Seed Company
   P. O. Box 548
   Navasota, Tex. 77868

   Rogers Delinted Cottonseed Company
   P. O. Drawer 1340
   Waco, Tex. 76703

   SEE
   Selective Educational Equipment, Inc.
   Three Bridge Street
   Newton, Mass. 02195

Note: Mention of a proprietary product in this publication does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its endorsement by the Department to the exclusion of similar products that may be suitable.

Experiment No. 1

Objective

To compare the growth of test seeds exposed to 5° C. temperature during initial water uptake with that of control seeds exposed to 31° C.

Procedure

1. Prepare the test group of seeds:
   a. Puncture the tops of 60 seeds with a dissecting needle to increase the permeability of the seed coats.
   b. Place two pieces of germination paper on top of a piece of wax paper. Pour 70 milliliters (ml.) of water at 5° C. over the two pieces of germination paper as shown in figure 5A. Fold back the top piece of germination paper as a cover sheet.
   c. Place 30 seeds (root points down) 1/2 inch apart on the germination paper in two rows as shown in figure 5B. Fold the germination paper as shown in figure 5C. Place a rubberband around the paper and stand it in a beaker containing a small amount of distilled water as shown in figure 5D.

2. Prepare the control group of seeds. Follow the same procedure as in step 1 except treat the germination paper with 70 ml. of water at 31° C.

3. Place the test group of seeds in the refrigerator at 5° C. (41° F. refrigerator temperature) for 24 hours. Then germinate the two groups of seeds (test and control) in the incubator at 31° C. (88° F.) for 3 days.

4. After 3 days germination, compare the growth and general condition of the two groups of seeds.

   As an option, you can determine seedling development by comparing the dry weight ratio of hypocotyl to total seedling of the test seedlings with that of the control seedlings. Cut the cotyledons from the hypocotyl-radicle of 15 test seedlings (fig. 6). Dry bulked parts at 55° to 60° C. (131° to 140° F.). Weigh both parts to obtain the dry weight ratio of hypocotyl-radicle to total seedling. Repeat the procedure with 15 control seedlings. Record and compare your findings.

5. Prepare a number of pots containing a nutrient soil mixture. Plant the two groups of seedlings (15 in each group if you used the optional method of determining seed development in step 4) in the pots. Mark the pots to show which contain test seedlings and which contain control seedlings. Do not expose the seedling roots to the drying effect of air for more than a few seconds.

6. Grow the seedlings for 2 weeks. During this time observe and record the height of the plant and the width of the first true leaf (not the cotyledon). At the end of the second week, gently uproot or wash out the seedlings and measure root growth. Record all measurements in centimeters. Compare the two groups of plants and observe any abnormalities in growth.

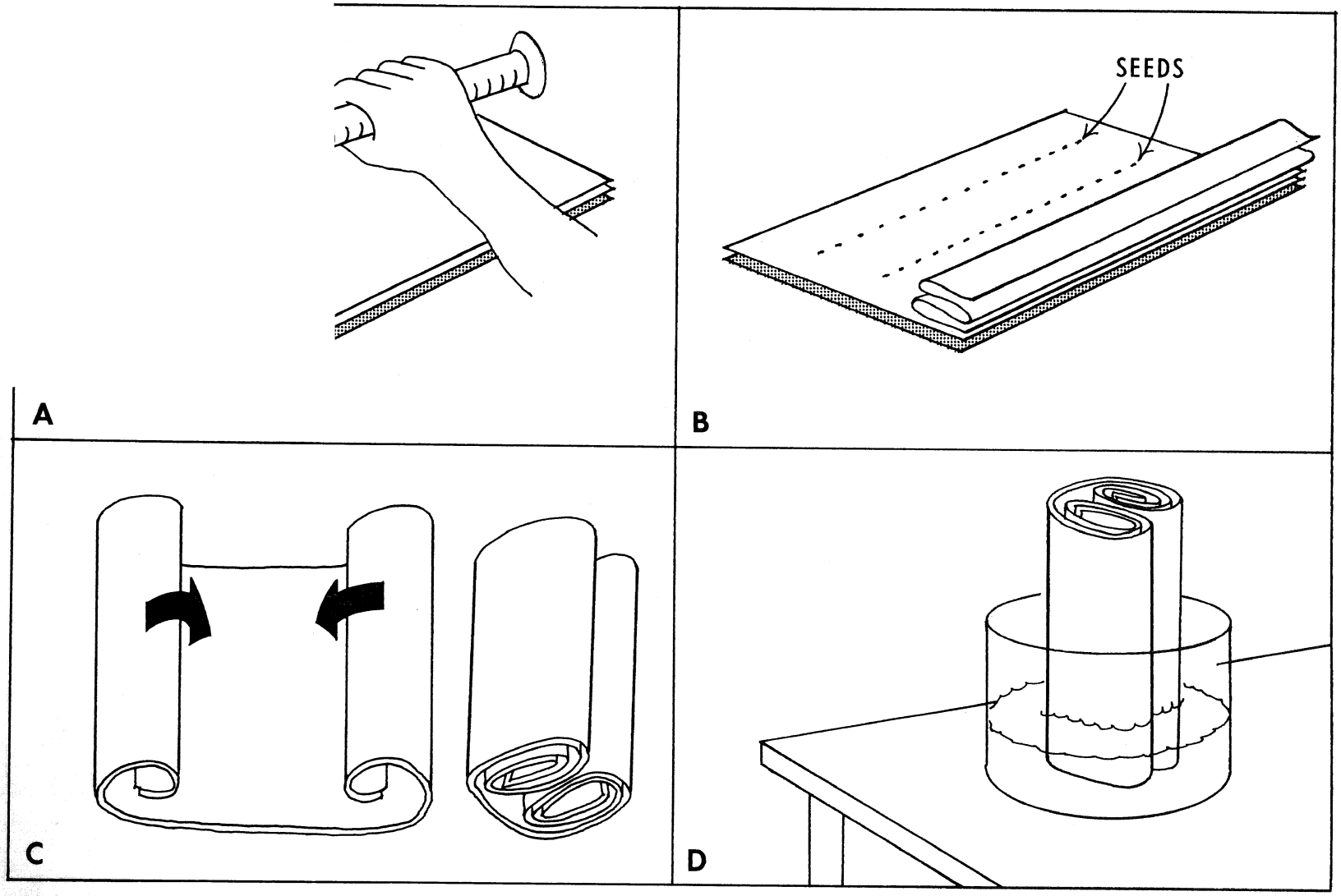


Figure 5.—Preparation of seeds for experiments.

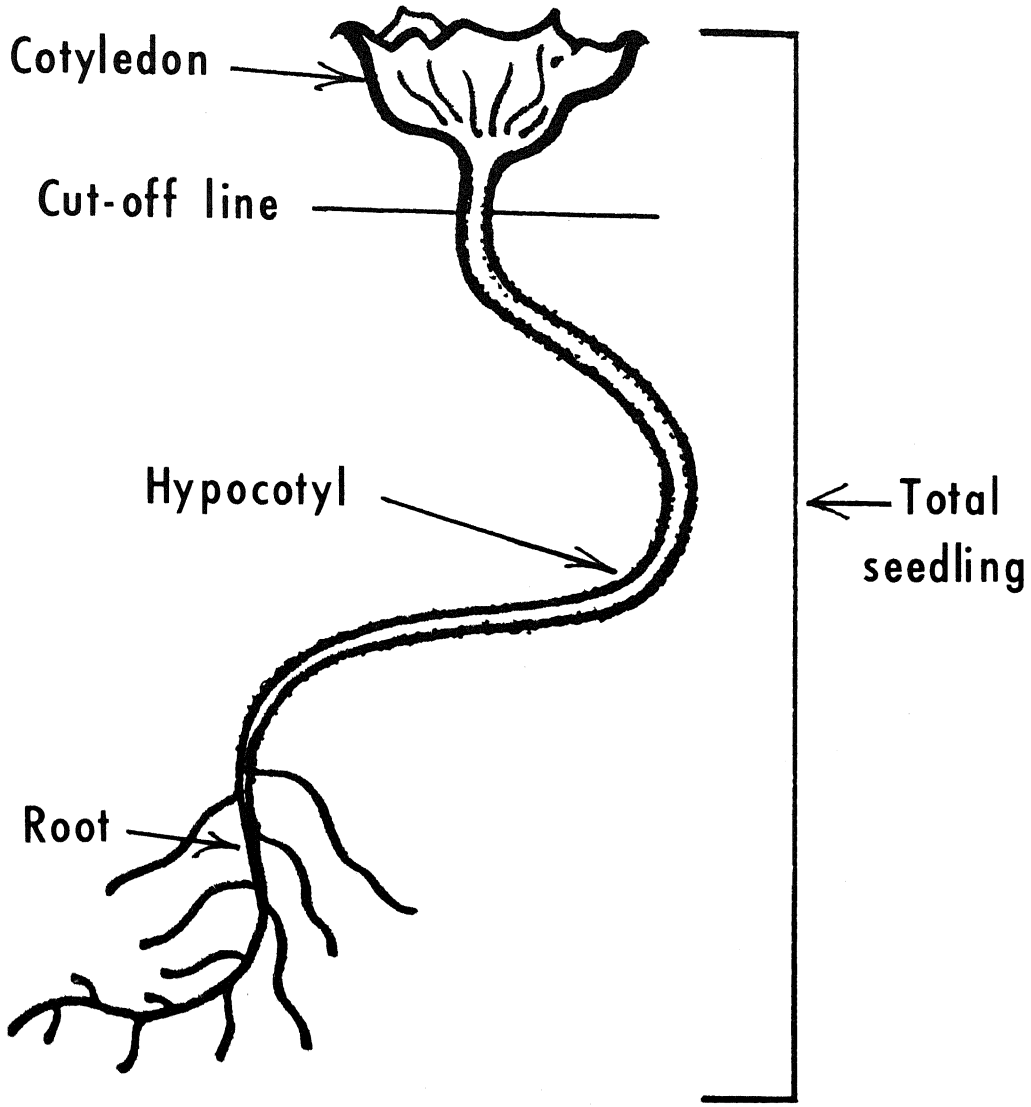


Figure 6.—Parts of seedling.

Experiment No. 2

Objective

To determine the effects of exposing seeds to 5° C. temperature for increasing periods of time during initial water uptake.

Procedure

1. Puncture 120 seeds as in experiment No. 1.

2. Divide the seeds into four groups of 30 seeds each and place each group into a 100 ml. beaker. Cover the seeds with distilled water that has been chilled to 5° C.

3. Place three of the beakers into the refrigerator at 5° C. Chill one for 8 hours, one for 16 hours, and one for 24 hours. The fourth beaker is not chilled in the refrigerator.

4. Germinate the seeds between germination paper, as in experiment No. 1, for 3 days.

5. Plant and grow the seedlings for 2 weeks or longer, and then evaluate their growth as in experiment No. 1

Experiment No. 3

Objective

To determine the stage of germination at which seeds are most susceptible to chilling damage.

Procedure

1. Puncture 120 seeds and divide them into four groups as in experiment No. 2.

2. Prepare the seeds in germination paper as in experiment No. 1.

3. Allow the four groups to germinate for different lengths of time—0, 12, 24, and 48 hours—at 31° C. before chilling at 5° C. for 48 hours. (The first group of seeds is chilled immediately.) Then germinate all seeds for a total of 72 hours at 31° C. before transferring to soil culture.

4. Continue growing the seeds for 2 weeks and observe the results as in experiment No. 1.

Experiment No. 4

Objective

To determine seedling development in relation to the amount of water absorbed during hydration (soaking).

Procedure

1. Puncture 150 seeds as in previous experiments and divide them into five groups of 30 each.

2. Weigh each group to obtain the dry weight.
3. Soak the five groups at 31° C. for different lengths of time—0, 1/2, 1, 2, and 4 hours. (The first group is not soaked.)

4. To determine the percentage of water absorbed after the hydration period, surface blot the seeds with tissue paper and reweigh them.

5. After the hydration period, place each group of seeds in germination paper as in previous experiments and chill for 48 hours at 5° C. Then germinate the seeds for 3 days at 31° C.

6. Relate any growth of the seedlings to the percentage of moisture absorbed.

BIBLIOGRAPHY

Standard biology text (for basic plant structure).

Christiansen, M. N. Influence of chilling upon subsequent growth and morphology of cotton seedlings, *Crop Science* 4:584-586.

Induction and prevention of chilling injury to radicle tips of imbibing cottonseed, *Plant Physiology,* 43 (May 1968), 743-746.

Periods of sensitivity to chilling in germinating cotton, *Plant Physiology,* 42 (March 1967), 4431-4433.

United States Department of Agriculture. A prescription for seed chilling injury, *Agricultural Research* (July 1968), 13.

Tharp, W. H. United States Department of Agriculture, The Cotton Plant, *Agricultural Handbook* No. 178, (June 1960).

Prepared by

Information Division
Agricultural Research Service

Washington, D. C. ☆ U.S. GOVERNMENT PRINTING OFFICE : 1971 O—448-323 Issued October 1971

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price 15 cents
Stock Number 0100-1568